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Clinical Use of Estradiol benzoate to Inhibit the Aggressive Behavior in Closed Breeder Calves

استخدام الاسترادايول بنزويت سريرياً لتثبيط السلوك العدواني في عجول التربية المغلقة

Gassan Hamdan Jameel
Diyala University/of Veterinary

Mohammed Saad Al-Muslehi
Diyala University/of Veterinary

Omer Jasim Katwan
Diyala University/of Medicine

Abdul-Wahab Abdul-Razaq Ijbara
Diyala University/Educational Razi

Abstract:

A present study was carried out to investigate the effects of Estradiol benzoate on aggressive behavior in male calves through studying the testosterone and estrogen hormones. Twenty calves were randomly divided into two groups (ten in each group) and treated as follows: calves in the first group considered as control without treated, whereas the calves in the 2nd group were injected Estradiol benzoate (0.03 mg/Kg) subcutaneously on the first day of experiment and termed as estradiol treated group, blood samples were collected on day 14 and day 28 of the experiment for measuring the hormones. Results revealed a significant increase ($P \leq 0.05$) in testosterone hormone of estradiol treated group as compared with the control group at day 14, whereas testosterone significantly decreased ($P \leq 0.05$) in of estradiol treated group when compared with the control at day 28. Furthermore, within group there was a significant ($P \leq 0.05$) decrease in testosterone at day 28 as compared with day 14. Moreover, estrogen hormone significantly increased ($P \leq 0.05$) in the estradiol treated group at all periods of experiment when compared with the control group. On conclusion it seems likely that subcutaneous injection of calves with (0.03mg/Kg) of Estradiol benzoate caused obvious decrease of testosterone and consequently inhibition of aggression in calves.

Key words: Aggression**,** Calves**,** Estradiol benzoate, Testosterone and Estrogen.

الخلاصة:

صممت هذه الدراسة لمعرفة تاثير الاسترادايول بنزويت على السلوك العدواني في العجول الذكور وذلك من خلال دراسة الهورمونات التالية: هورموني التيستيستيرون والاستروجين. تم استخدام 20 من عجول التربية المغلقة وقسمت عشوائيا الى مجموعتين وبواقع عشرة حيوانات لكل مجموعة وعوملت كالاتي: حيوانات المجموعة الاولى عوملت على انها مجموعة سيطرة بدون معاملة، بينما حيوانات المجموعة الثانية اعطيت 0.03 ملغم/كغم تحت الجلد في اليوم الاول من التجربة وسميت بالمجموعة المعالجة بالاسترادايول. سحبت عينات الدم في الايام 14 و 28 من فترة التجربة لغرض تقييم الهورمونات. اظهرت النتائج ان معدل هورمون التيستيستيرون قد ازداد وبشكل معنوي ($P \leq 0.05$) في المجموعة المعاملة بالاسترادايول مقارنة بمجموعة السيطرة في اليوم 14 من التجربة. بينما انخفض هورمون التيستيستيون بشكل معنوي ($P \leq 0.05$) في المجموعة المعاملة بالاسترادايول بالمقارنة مع مجموعة السيطرة في اليوم 28. بالاضافة لذلك, يوجد في نفس المجموعة المعالجة بالاسترادايول انخفاض معنوي ($P \leq 0.05$) في هورمون التيستيستيرون في اليوم 28 من التجربة بالمقارنة مع اليوم 14. علاوه على ذلك, هرمون الاستروجين ازداد وبشكل معنوي ($P \leq 0.05$) في المجموعة المعالجة بالاسترادايول بالمقارنة مع مجموعة السيطرة في جميع فترات التجربة. نستنتج من ذلك بأن حقن الاسترادايول بجرعة 0.03 ملغم/كغم تحت الجلد تسبب بانخفاض هورمون التيستيستيرون وتبعاً لذلك تم تثبيط العدوانية في العجول.

الكلمات المفتاحية: العدوانية, العجول, الاسترادايول بنزويت, التيستيستيرون والاستروجين.

Introduction:

Aggression is defined as recurrent violent events, either verbal or physical in nature, that are out of proportion to the precipitating stress or provocation and that stem from organic etiologies or it is a response that delivers noxious stimuli to another person" (1). In animals, there are various

forms of aggression classified into predatory, intermale, fear-induced, irritable, territorial, maternal and instrumental (2). Androgens have been found to affect only certain forms, for example, intermale aggression (3). The classical hormone removal and replacement experiment approach has shown a definite link between testosterone and aggression in animals. In general, castration leads to a decrease in aggression whilst replacement of testosterone restores the aggression behavior. Different regions in the brain modulate different hormone-dependent aggression (4,5,6). In 1993, intermale aggressive behavior in rats induced by electrical stimulation of the hypothalamus was enhanced by androgens (3). Studies in rats, mice, monkeys and humans show that competitive or intermale aggression increases at puberty, whereas it is reduced by castration and increased by testosterone injection (6,7,8).

Aggression in females, like in males, appears to be facilitated by testosterone in a dose-dependent manner (6). In one study, ovariectomized female rats were given daily injections of testosterone and estradiol. It was found that testosterone increased aggressiveness (9,10). However, the presence of testosterone was not sufficient to 'activate' the aggression. Instead, the missing essential factor was likely to be an experiential event, for example, competition. Therefore it is possible that hormones are required for its persistence but are not sufficient to activate the neural circuitry required for aggression.

Materials and Methods:

Twenty male calves were used in this study, the age of these animals ranged between (15-18) months and weight was about (130-200) kg. Calves were kept under suitable environment conditions, all calves were allowed to free access of drinking water and basal diet.

Calves divided randomly in to two groups (10) calves for each group and handled as follows: (I) Control group: Animals of this group fed on basal diet and ordinary tap water. (II) estradiol treated group: calves fed on basal diet and ordinary tap water and injected with 0.03mg/Kg estradiol benzoate (Intervet pharmaceutical, Holland) subcutaneously, the dose was approved after making several experiments until reach it.

Blood samples were collected two times, once after 14 days of the experiment and the other at the end of the experiment (28 days), the samples blood were refrigerated, centrifuged at (3000 rpm).

The hormonal assay of testosterone and estrogen were estimated in the serum samples performed by solid phase radioimmuno-assay using standard kits.

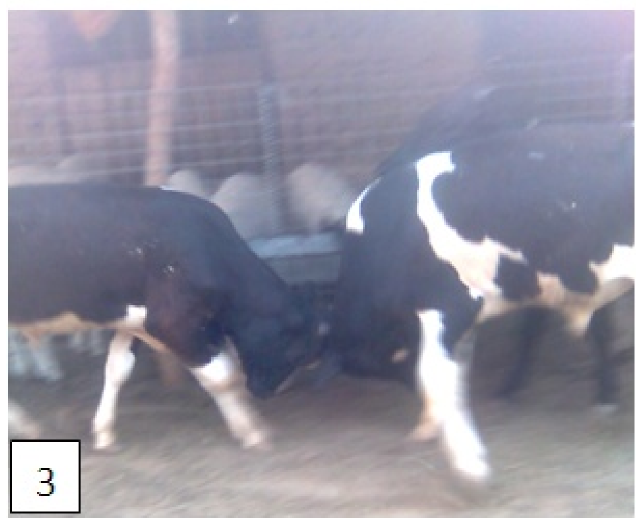
Results are expressed as mean ± SE. statistical analysis of data was performed on the basis of two- way analysis of variance ANOVA II. Group differences and within group differences were determined using least significant difference (LSD) test at ($P<0.05$) (11).

Results:

Pictures: (1,2and3) represents different forms of aggression (fighting and mounting), picture 4 shows many lesions resulting from aggression (fighting or mounting). These pictures had been taken during the first period of experiment.









The data of table (1) referred to a significant increase (P≤0.05) in testosterone of estradiol treated group as compared with the control group at day 14, whilst the results in the same table showed significant decrease (P≤0.05) in testosterone of estradiol treated group as compared with the control group at day 28. Furthermore, within group there was a significant (P≤0.05) decrease of the mean of testosterone at day 28 as compared with other time (day 14).

Table (1): Testosterone hormone in calves, normal (control) and after treated with estradiol benzoate.

Group / Time	Control group (mean ± SE) ng/ml	Estradiol treated group Testosterone (mean ± SE) ng/ml
Day 14	2.950 ± 0.014 B b	5.490 ± 0.173 A a
Day 28	3.324 ± 0.017 B b	1.423 ± 0.002 C c

- L.S.D= 1.104
- Values are presented as means ±SE (n= 10 calves/group).
- (Estradiol treated group) group given estradiol benzoate (0.03 mg/kg B.W).
- The different capital letters denote significant differences between groups (P≤0.05).
-The different small letters denote significant differences within group (P≤0.05).

Estrogen hormone in (table: 2) was significantly increased (P≤0.05) in the estradiol treated group when compared with the control group at all periods of experiment. Furthermore, within groups (table: 2), the results showed no significant differences in estrogen hormone in estradiol treated group and control group at all periods of experiment.

Table (2): Estrogen hormone in calves, normal (control) and after treated with estradiol benzoate.

Group / Time	Control group (mean ± SE) ng/ml	Estradiol treated group Estrogen (mean ± SE) ng/ml
Day 14	3.537 ± 1.068 A a	7.120 ± 0.254 B b
Day 28	3.498 ± 1.062 A a	6.846 ± 0.140 B b

- L.S.D= 1.315
- Values are presented as means ±SE (n= 10 calves/group).
- (Estradiol treated group) group given estradiol benzoate (0.03 mg/kg B.W).
- The different capital letters denote significant differences between groups (P≤0.05).
-The different small letters denote significant differences within group (P≤0.05).

Discussion:

Changes in the concentration of hormones may have profound effects on mood and behavior in animals. Variations in hormone levels have been implicated in some disorders such as aggression (12,13). Our data indicated that testosterone hormone caused stimulation of aggression this came compatible with (14) who established that gonadal steroid hormones, by acting on the central nervous system, regulate various neuroendocrine events related to reproduction and reproductive behaviors in both sexes. In males, testosterone is a major hormone that facilitates both sexual and aggressive behaviors. The cause of aggression in calves perhaps due to the testosterone hormone has been elevated. The mechanisms underlying the behavioral effects of testosterone, however, are complicated by the fact that endogenous testosterone can act not only through the androgen receptor (AR) as can testosterone metabolite (5a-reduced dihydrotestosterone), but also can act through estrogen receptors (ER) after being aromatized to estradiol in the target tissues including the brain (15) this mechanism probably explains why the estrogen hormone has been elevated in calves.

On the other hand, increased testosterone hormone level led to aggression in calves probably because testosterone acts as a prohormone which when converted into 5-alpha-dihydrotestosterone (5a-DHT) acts on androgen receptors or when converted into estradiol by the enzyme aromatase, acts on estrogen receptors. There is overwhelming evidence that most of the effects of testosterone in mediating aggression occur after aromatization (16). For example, testosterone induced aggression is concurrent with an elevated level of aromatization and nuclear estrogen receptor activity in the hypothalamic/preoptic area. Treatment with an aromatase inhibitor blocked this aggression and lowered nuclear activated estrogen receptors (17). Furthermore, the intensity of aggressive behavior was directly correlated with the aromatase activity in the posterior hypothalamus (18).

Moreover, the lipophilic nature of testosterone may be enabling it to easily pass through the blood–brain barrier in free form (not bound to protein). At the cerebral level, steroid hormones originating from the periphery influence the function of nerve cells dispersed throughout the body (19). The best example of this influence is the neurons that secrete hypophysiotrophic factors stimulating the production of pituitary hormones such as ACTH and gonadotropins. These hormones are subjected to regulation by the corresponding steroid hormones by a feedback mechanism (20). Testosterone and estrogen receptors are primarily located in the limbic areas of the brain. Many studies in animals and humans have found that rage reactions occur with stimulation of

limbic structures but not as the result of stimulation of the neocortex (19). Phylogenetically and histologically, the limbic gray matter is clearly more primitive than the other cortical areas. Animal with high testosterone have diminished higher cortical function that may lead to a decrease in the psychosocial or environmental influences on behavior (20). Therefore, the animal with high testosterone may be more influenced by primitive or limbic impulses and may lack the inhibitions of learned psychosocial behaviors. This explanation is consistent with the hypothesis that deeper limbic structures contain the template for aggressive behavior but that higher centers, when intact, maintain control of aggression.

Estrogens have powerful effects on behavior in a variety of contexts. These effects perhaps mediated by the classical estrogen receptors (ER) such as ER_ as well as membrane receptors such as G protein-coupled receptor (21, 22). The effects of estrogen signaling on behavior are dependent on the environment. Several forms of endocrine disruptor compounds have been identified that can directly or indirectly alter estrogen signaling (23,24). The phytoestrogen isoflavone, which is present in many animal feeds, inhibits ER expression in the brain and inhibits female sexual behavior (25).

Nonetheless, our data indicate a specific association between aggressive behavior and testosterone and suggest that trials to test novel treatment approaches for physically aggressive calves (e.g., lowering testosterone levels) should be undertaken. On conclusion it seems likely that subcutaneous injection of calves with (0.03mg/Kg) of Estradiol benzoate caused obvious decrease of testosterone and consequently inhibition of aggression in calves.

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